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BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE

FOREST INSECT INVESTIGATIONS

RESULTS OF QUANTITATIVE ANALYSES OF WOOD SAMPLES FROM TREES INJECTED WITH HEAVY DOSES OF COPPER SULFATE

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Forest Insect Laboratory
Coeur d'Alene, Idaho
Jan. 22, 1938

Dr. F. C. Craighead
Washington
D. C.

Dear Dr. Craighead:

There is enclosed Mr. Bedard's report "Results of Quantitative Analyses of Wood Samples from Trees Injected with Heavy Doses of Copper Sulfate". Though a copy has been sent to Mr. Wilford, we are enclosing an extra copy for distribution as you may see fit.

The quantative analyses of the wood samples from trees injected in 1936 were made at the Asheville, North Carolina, station, and I wish to take this opportunity of expressing our appreciation for this cooperative service.

We should appreciate your comments and suggestions relative to the material discussed within this report. We are quite interested in what appears to be a period of low mortality which occurs in trees which are treated approximately 30 days after attack. We are somewhat at a loss to explain this occurrence, but feel that perhaps tree moisture may play a rather important role. Trusting we may have the benefit of your discussion of this report, I remain

Respectfully yours,

James C. Evenden
Entomologist

cc to:
Mr. Miller
Mr. Keen
Mr. Beal
Mr. Wilford

RESULTS OF QUANTITATIVE ANALYSES OF WOOD SAMPLES FROM TREES INJECTED WITH HEAVY DOSES OF COPPER SULFATE

Western white pine trees injected with commercial doses of copper sulfate during 1936 were examined in the spring of 1937 to determine the mountain pine beetle mortality secured by chemical treatment. At the time of this examination, increment cores were taken from two sides of each tree at 20-foot intervals starting at 5 feet. Cores from 41 of these trees were sent to the Asheville, North Carolina laboratory where they were tested quantitatively for the presence of copper sulfate. The outer inch of each core was divided into four, quarter inch pieces, each quarter inch being tested separately so that data are available relative to radial penetration as well as vertical distribution. This report attempts to analyze the results of these tests.

General Discussion of Distribution

In general, the distribution of copper shown by the core analyses is very peculiar, particularly when it is compared with brood mortality resulting from the injection. For example, three trees in which 100 percent mortality was secured showed no recoverable copper when the cores were tested. Conversely, two trees in which there had been no apparent mortality showed at least .11 pound of copper in the outer quarter inch for the total infested length, in one case up to 100 feet. This peculiar distribution is shown in table I in which distribution is compared according to mortality.

Table I

AVERAGE DISTRIBUTION OF COPPER ACCORDING TO MORTALITY

Height	Depth 1[a]	2	3	4
	Mortality 0-25%			
5	.30[b]	.18	.14	.09
20	.16	.08	.05	0
40	.09	.02	.04	.02
60	.02	.02	.02	0
80	.08	.06	.06	.06
100	.05	.05	0	0
	Mortality 26-50%			
5	.59	.45	.31	.14
20	0	0	0	0
40	.02	0	0	0
60	0	0	0	0
80	0	0	0	0
	Mortality 51-75%			
5	1.18	.61	.16	0
20	0	0	0	0
40	0	0	0	0
60	0	0	0	0
	Mortality 76-100%			
5	.33	.22	.14	.10
20	.05	.01	0	.01
40	.04	.03	.01	.01
60	.08	.04	.01	.02
80	.07	0	0	0
100	.03	.03	0	.03

[a] Depth of penetration shown by quarter inches, 1 = outer quarter inch.

[b] Copper shown in pounds per cubic foot of wood.

It is quite apparent in table I that distribution of the poison in no way helps to explain the mortality which was secured, because best average distribution occurred in the group showing poorest mortality. For the purpose of comparison, table II shows the average

diameter, blue stain, age of attack, and growth rate for each of the groups in table I.

Table II

AVERAGE CONDITION OF TREES IN EACH MORTALITY GROUP

Percent mortality	0-25	26-50	51-75	76-100
Diameter	24.8"	19.1"	17.3"	19.6"
Blue stain	Medium	M+	M+	M
Age of attack	49 days	59	58	61
Rings last ½ inch	16	21	40	16

Diameter, of course, should not be a factor determining distribution because the dosage of poison was varied according to the size of the tree. Blue stain averaged approximately the same in all groups, and the same average rate of growth was present in the lowest and highest mortality groups. The time elapsing between attack and injection therefore is the only significant variable, and although this could be discounted on the fact that its real importance is in governing blue stain development, it is interesting to note that there was a longer average elapsed time in the group showing the highest mortality.

One fact is apparent in all analyses, however, which is that a much heavier concentration of poison is present immediately above the collar than in any other portion of the tree. Table III shows the average distribution of the chemical in all trees.

Table III

AVERAGE DISTRIBUTION OF CHEMICAL IN ALL TREES

Height	Depth 1	2	3	4
Ft.				
5	.39	.26	.16	.09
20	.07	.02	.008	.005
40	.04	.02	.02	.01
60	.05	.02	.01	.01
80	.07	.03	.03	.03
100	.04	.04	0	.02

It will be remembered that in attempting to explain the poorer results obtained in the 1936 injections, the thought was advanced that in using the saturated solutions necessary for such heavy dosages there was a mechanical blocking of the tracheids resulting from a crystallization of the copper sulfate near the point of injection. As shown in table III, this is apparently what occurred. In some trees, the crystallization undoubtedly took place in the saw-kerf itself before the poison entered the tree, while in others it occurred in the tracheids just above the point of injection, as shown by the heavy concentration of poison at 5 feet.

Distribution According to Blue Stain and Age of Attack

If, as indicated, the copper sulfate blocked conduction in cases of slow ascension of the poison, the slower movement resulted in all probability from previous blocking by blue stain. Table IV shows the distribution of copper according to the intensity of blue stain at the point of injection.

Table IV

DISTRIBUTION OF COPPER ACCORDING TO BLUE STAIN

Height	Depth 1	2	3	4
Heavy Blue Stain--Mortality 77.8%				
5	.35	.23	.16	.10
20	.05	.01	0	0
40	.02	.03	.02	.01
60	.03	.02	.01	.02
80	0	0	0	0
100	0	0	0	0
Medium Blue Stain--Mortality 73.6%				
5	.53	.35	.23	.14
20	.01	.02	.01	.01
40	.02	.01	0	0
60	.02	0	0	0
80	.11	0	0	0
Light Blue Stain--Mortality 47%				
5	.29	.22	.06	.02
20	.02	.02	.02	0
40	.11	.02	.05	.02
60	.05	.05	.05	0
80	.22	.16	.16	.16
100	.05	.05	0	0
No Visual Blue Stain--Mortality 99.3%				
5	.18	.07	.04	0
20	.26	.07	0	0
40	.18	.07	.03	.03
60	.22	.11	0	0

Table IV shows that there was not only a tendency toward radial rather than vertical distribution in the more heavily blue-stained trees, but that distribution tended to be more even in the lightly blued treesor trees which showed no visual blue stain at all. It is quite apparent that best vertical distribution was secured in unblued or lightly blued trees.

Table V shows the distribution of copper according to the age of attack.

Table V

DISTRIBUTION OF COPPER ACCORDING TO AGE OF ATTACK

Height	Depth 1	2	3	4
30 Days Old or Less--Av. Mortality--72.1%				
5	.33	.22	.04	.02
20	.15	.05	.02	0
40	.11	.04	.02	.02
60	.18	.11	.03	0
80	.11	.11	.22	.22
100	.11	0	0	0
31-50 Days Old--Av. Mortality--57.1%				
5	.58	.37	.22	.13
20	.10	.03	.02	.02
40	.06	.01	.02	.01
60	0	0	0	0
80	.16	.11	.05	.05
100	0	.11	0	0
51-70 Days Old--Av. Mortality--84.9%				
5	.14	.09	.04	.02
20	.03	0	0	0
40	.03	.03	0	0
60	.11	.03	.03	.03
80	.11	0	0	0
100	.11	.11	0	0
Over 71 Days Old--Av. Mortality--79%				
5	.49	.33	.25	.16
20	.07	.02	0	0
40	.02	.02	.02	.01
60	.01	.01	0	.01
80	0	0	0	0
100	0	0	0	.05

Table V shows that in general more erratic distribution occurred in those trees which had been attacked for a longer period of time. If,

as believed, the poorer distribution in older attacks results from greater blue-stain development, it is evident from the uneven distribution shown in table V that age of attack is not the only factor governing this development. In all probability other factors, such as intensity of inoculation, nutrition, solar heat and wood density, accelerate or retard it sufficiently to cause uneven development and consequent uneven distribution of the copper.

There is in addition one other circumstance which must be considered under any discussion of age of attack; namely, that there has been evident, during all the years of this study, a "low mortality period" which occurs soon after the tree has been attacked. We have always recognized the fact that lower mortalities occur just prior to the time that attacks become too old for successful injection. This new low period occurs earlier, however, approximately thirty days after the attack of the tree. This statement does not intend to imply that all unsuccessful injections occur within one definite age period, because trees with low mortality are to be found in nearly all age groups. There is, however, a definite increase in the frequency of such trees until maximum abundance is reached in one age period, following which there is an irregular decline in their abundance. The following four tables demonstrate this fact. Table VI shows the average mortality by 10-day age periods during the last four years of the study, when large numbers of trees were treated annually.

Table VI

AVERAGE MORTALITY ACCORDING TO AGE OF ATTACK DURING LAST FOUR YEARS

Year	Age of attack in days									
	1-5	6-15	16-25	26-35	36-45	46-55	56-65	66-75	76-85	86-95	96-105
1933		90	90	90	91	78	96	88	86	46	67
1934	100	92	73	42	80	68	62	84		5	
1935		99	95	99	87	96	86	94		85	40
1936				76	85	53	92	78	77	84	
Average for all years	100	98	90	84	88	81	89	88	83	70	52
Total number of trees	4	99	85	59	93	79	53	90	22	19	9

As would be expected, mortalities in the higher age groups are somewhat variable and tend to be low generally. There is, however, a marked low period of mortality long before the age limit of successful injection is reached. In 1933 and 1936 this low point occurred when attacks were from 46 to 55 days old, in 1934 when attacks were 26 to 35 days old, and in 1935 when 36 to 45 days had elapsed after attack.

In table VII the trees have been divided into three groups to show average mortalities prior to, during, and following the low mortality period.

Table VII

AVERAGE MORTALITY PRIOR TO, DURING, AND FOLLOWING LOW MORTALITY PERIOD

Year	Average mortality prior to low period		Average mortality during low period		Average mortality following low period	
		No. trees		No. trees		No. trees
1933	90	96	78	33	87	105
1934	85	29	42	11	73	34
1935	98	152	87	29	90	74
1936	80	14	53	7	83	28
Average all years	93	291	74	80	85	241

The most striking feature of table VII is that in spite of the low mortalities which occur normally in the highest age groups as shown in table VI the average mortality following the low period is decidedly higher than that attained during the low period, and in fact, during 1936 the average kill is even higher following the low period than prior to it.

Undoubtedly these figures would be even more striking if all low mortality trees could be segregated within one age group. As mentioned, however, there is an increase, peak, and irregular decrease in their abundance throughout the age range, thus lowering average mortalities prior to and following the low period. Table VIII shows the frequency of trees with mortality below 75 percent in the various age of attack groups.

Table VIII

NUMBER OF TREES IN EACH AGE GROUP WITH MORTALITY BELOW 75 PERCENT

Year	Age of attack in days									
	1-5	6-15	16-25	26-35	36-45	46-55	56-65	66-75	76-85	86-95	96-105
1933		0	5	2	5	9	8	3	3	4	2
1934	0	1	3	8	2	4	4	3		1	
1935		0	3	0	4	1	2	1	0	0	3
1936				2	1	4	0	2	2	1	
Total low mortality trees	0	1	11	12	12	18	14	9	5	6	5
Total number of trees	4	99	85	59	93	79	53	90	22	19	9

In table VIII it is apparent that the greatest abundance of low mortality trees occurred each year during the age period showing lowest mortality (table VI). It is also obvious that this frequency is not dependent upon the frequency of sampling except perhaps in the older age groups.

In table IX the age groups have been shifted somewhat to the right or left so that the 10-day periods showing lowest mortality are in the same column. In this manner the 10-day age groups immediately preceding and following the low period can be compared.

Table IX

FREQUENCY OF LOW-MORTALITY TREES PRIOR, DURING, AND FOLLOWING LOW MORTALITY PERIOD

Year	10-day Periods										
	Before low period				Low period	After low period					
1933	0	5	2	5	9	8	3	3	4	2	
1934		0	1	3	8	2	4	4	3		1
1935		0	3	0	4	1	2	1		0	3
1936			2	1	4	0	2	2	1		
Total low mortality trees	0	5	8	9	25	11	11	9	8	2	4
Total trees	1	122	74	94	80	72	85	46	23	9	6

Table IX shows that when the low periods are made to coincide and the numbers of low-mortality trees in each age group are totalled in this manner, there is a gradual increase in abundance prior to the low period, with a gradual decrease following maximum abundance.

It is apparent therefore that there are two periods when

the injection method of control is not particularly successful. One of this occurs approximately three months after the tree has been attacked and undoubtedly results from complete obstruction of the tracheids by blue stain hyphae. This low-mortality period has always been recognized since injection in white pine was first begun and is of little significance because in any actual control work most of the infested trees will have been injected before this period is reached. The other low-mortality period, however, is of considerably more importance because it occurs approximately midway in the successful injection period and thus may jeopardize the use of injection as a control method.

What causes this low mortality period is not known at this time but it is obvious that considerable study should be given it in the future. Its occurrence in different age groups during different years would indicate that it is affected by climate, while its gradual increase and decrease in prevalence would indicate that it is a temporary condition within the tree itself. Beyond this, however, I can offer but three possible theories: (1) Maximum vegetative growth by blue stain fungi in the tracheids, (2) temporary saturation of the tree, and (3) change in gradient direction.

The first theory is rather weak, in that it implies maximum vegetative growth by blue stain fungi in the tracheids, with a subsequent decline in volume when the fungus has become established generally throughout the sapwood. It is logical to assume that this growth does occur more rapidly in the unobstructed vessels than it does in the remainder of the sapwood because of the lower resistance,

but the theory must further imply that the volume of blue stain hyphae in the tracheids decreases soon afterward and that the final cause of unsuccessful injection must therefore be the collapse or breaking away of the tracheids themselves. Whether the blue stain fungi use passages of least resistance for temporary hyphae in order to establish themselves more generally, and how soon do tracheids collapse or separate at their junctures are the questionable factors in this theory.

The second theory is somewhat more sound in that it is based on one fact, which is that the sapwood of insect-killed trees becomes quite wet at one time following beetle attack. In all probability, this saturation point is reached as a result of the slowing up of transpiration and the continuation of root function at the same rate. This does not necessarily imply any great drying of the leaves, because the volume of water used by the tree is so great that even the slightest change would be sufficient. The leaves, however, do not decrease their functions until the water supply diminishes, and this necessarily implies some obstruction in the tracheids. It is possible that there is sufficient blue stain development to cause a retardation in water conduction resulting in a slight drying of the foliage, which is followed in order by saturation of the wood, cessation of root function, and decrease in water content until it is again possible for the slow ascension of water.

The third theory is based on the physiological principle that solutions travel within plants along definite gradients, that is from

points of one concentration to points of another concentration. Thus, it is entirely possible that following the stimulus of beetle attack the gradients are changed in an effort to overcome the attack, and that for a short time the flow of copper sulfate may be downward or inward rather than upward.

These of course are merely theories which are expressed in the hope that they will suggest additional possibilities. It is believed, however, that there is sufficient evidence of a low mortality period at a critical time to warrant considerable study before tree injection is adopted generally as a control method in western white pine. In fact, if the causes were completely understood, it is believed that more rapid advancements could be made in this study.

Distribution According to Rate of Tree Growth

The data secured from the 1935 injections indicated that the slower a tree had grown the greater would be the success of injection. This indication, however, was not borne out by the 1936 injections, but it will be remembered that no very good correlations were secured with any factor from this series of trees. Table X shows the distribution of copper according to the number of rings in the outer half inch of sapwood.

Table X

DISTRIBUTION OF COPPER ACCORDING TO RATE OF TREE GROWTH

Height	Depth 1	2	3	4
	6-10 Rings	Mortality--76%	Age Attack--63	
5	.27	.18	.11	.11
20	.08	.008	.008	0
40	.05	.03	.02	.01
60	.07	.04	.01	0
80	.05	.02	.08	.05
100	.11	.05	0	0
	11-15 Rings	Mortality--78%	Age Attack--69	
5	.44	.21	.12	.05
20	.01	0	0	0
40	0	.01	0	0
60	.03	.01	.01	.03
80	0	0	0	0
100	0	0	0	0
	16-20 Rings	Mortality 82%	Age Attack--64	
5	.64	.47	.37	.22
20	.09	.03	0	.02
40	.08	.04	.05	.02
60	0	0	0	0
80	.16	.11	.05	.05
100	0	.05	0	.05
	21-51 Rings	Mortality--60%	Age Attack--46	
5	.32	.24	.10	.02
20	.15	.05	.02	0
40	.06	.01	0	0
60	.05	.02	0	0
80	0	0	0	0

Table X shows that in the first three growth groups the age of attack remained fairly constant, but that mortality increased as the rate of growth decreased. It will also be noted that in these three groups the volume of copper deposited immediately above the point of injection is greater as the rate of growth decreases. In

the fourth group, the average age of attack falls within the low mortality period shown in table VI and thus shows a much lower average mortality than the other three groups, even though the attacks were much younger.

Summary

1. This report deals with the results of quantitative analyses of cores taken from western white pine trees which had been injected with heavy doses of copper sulfate.

2. In general, the distribution of copper is very peculiar and does not correspond with the brood mortality secured.

3. The average distribution of copper sulfate in all trees indicates that there was a heavy deposition of copper immediately above the point of injection, probably as a result of using saturated solutions. This heavy deposit probably blocked the tracheids and resulted in poor distribution throughout the rest of the tree.

4. Indications are that there is a tendency toward radial rather than vertical distribution in more heavily blue-stained trees, and that distribution is more even in lightly blue-stained trees.

5. Averages of data for the years 1933 to 1936 inclusive indicate that there is not only a maximum age of attack limit, beyond which injection is not successful, but that there is another low mortality period occurring approximately midway in the period of successful injection.

6. The data indicate that higher mortality and poorer distribution result in the more slowly growing trees.

Respectfully submitted,

W. D. Bedard
Associate Entomologist

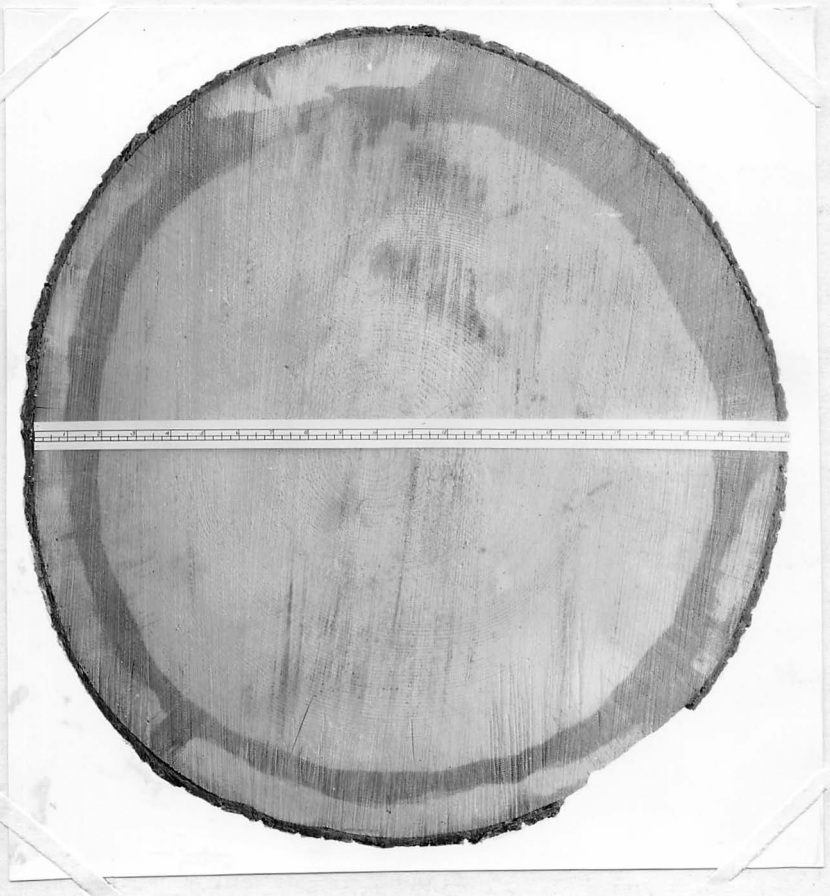

Fig. 1 - Cross section of tree 16 at 22 feet showing patches of clear, unblued sapwood resulting from presence of copper sulfate. Note the blue stain development inside the unblued areas. Tree diameter--30 inches. Age of attack 38 days. Injected with 12 pounds copper sulfate.

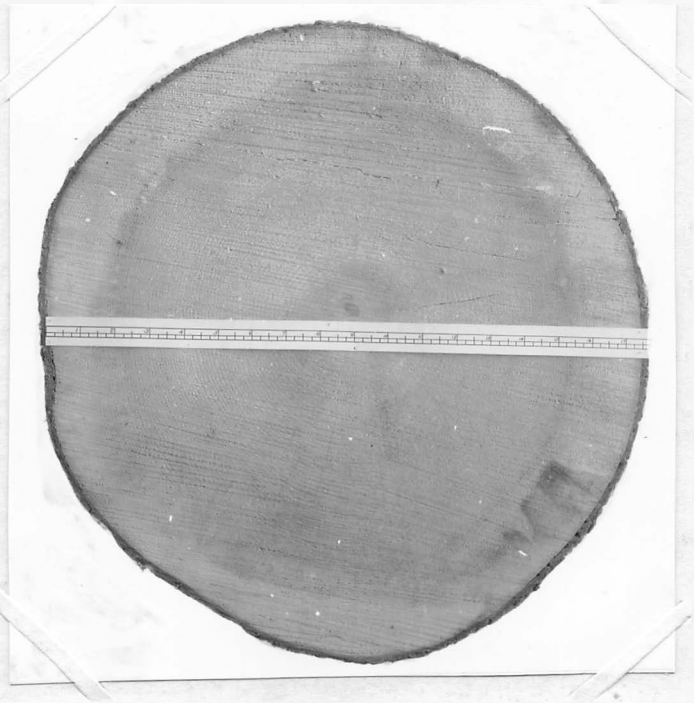

Fig. 2 - Cross section of tree 18 at 65 feet showing total absence of blue stain. Dark spot at lower right is light green in color, being heavy deposit of copper sulfate.

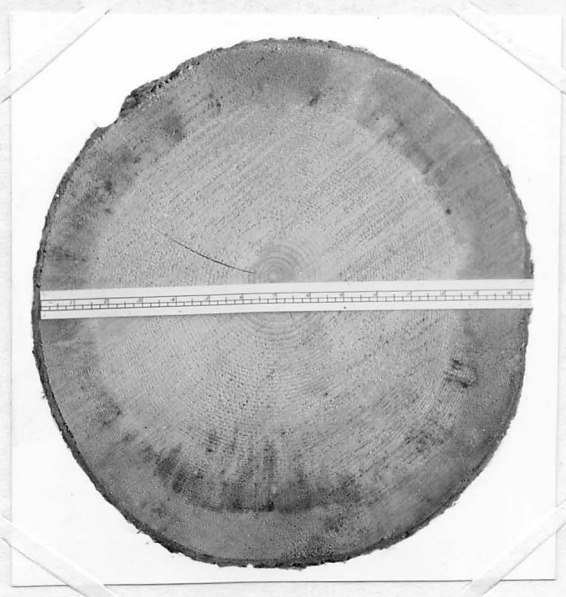

Fig. 3 - Cross section of tree 18 at 81 feet showing spotted appearance of blue stain and darker areas which are heavy deposits of copper sulfate.

Fig. 4 - Cross section of tree 624 at 71 feet showing normal development of blue stain.

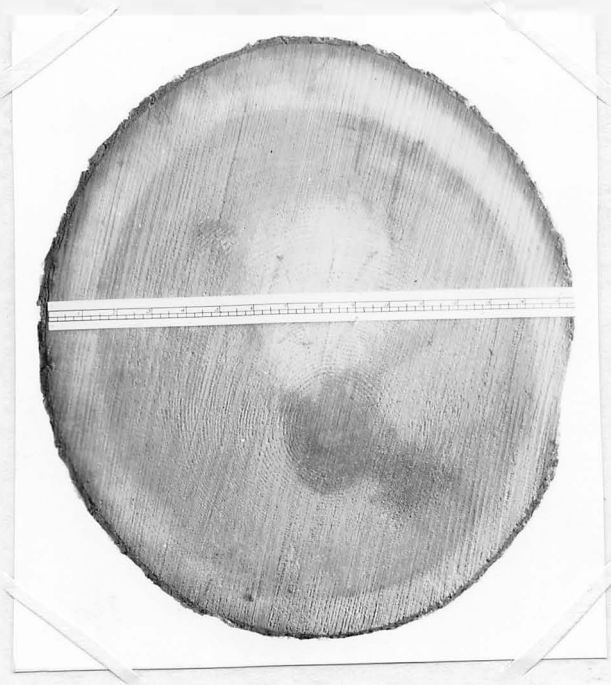

Fig. 5 - Cross section of tree 17 at 20 feet showing total absence of blue stain. Dark area just inside bark is red-brown stain apparent in all trees injected with ammonium flouride. Sapwood colored pale pink. Age of attack--30 days.